



QUALITY CONTROL for MOLECULAR DIAGNOSTICS

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Final Report

**QCMD 2009**

# HIV-1 Pro-viral DNA (HIVDNA09) EQA Programme

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The QCMD programme is organised  
in collaboration with the European  
Society for Clinical Virology and the  
European Society for Clinical  
Microbiology & Infectious Diseases.



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## 1. Programme aim

The primary aim of this External Quality Assessment (EQA) Programme was to assess the proficiency of laboratories in the detection of HIV-1 Pro-viral DNA.

## 2. Programme details

**Table 1: Programme details**

| HIVDNA09  |            |
|---|------------|
| Date of panel distribution                                | 23/03/2009 |
| Number of participants                                    | 58         |
| Number of countries                                       | 23         |
| Number of respondents                                     | 50 (86%)   |
| Number of datasets submitted                              | 54         |
| Number of qualitative datasets submitted                  | 46 (85%)   |
| Number of qualitative and quantitative datasets submitted | 8 (15%)    |

Eight people did not return results. Two of these officially withdrew citing the following reasons 'technical issue' (n=1) and 'internal issue' (n=1).

## 3. Panel composition

This EQA panel for the detection of HIV-1 Pro-viral DNA consisted of six samples containing various concentrations of HIV-1 Pro-viral DNA and two samples negative for HIV-1 Pro-viral DNA.

Peripheral blood mononuclear cells containing pro-viral HIV-1 subtype B virus, derived from clinical samples.

**Table 2: Panel composition**

| Sample      | Sample* content         | Sample † matrix | Sample conc. Copies/sample | Sample status   |
|-------------|-------------------------|-----------------|----------------------------|-----------------|
| HIVDNA09-01 | HIV-1 DNA Negative PBMC | Buffer          |                            | Negative        |
| HIVDNA09-02 | HIV-1 DNA PBMC          | Buffer          | 20                         | Strong Positive |
| HIVDNA09-03 | HIV-1 DNA Negative PBMC | Buffer          |                            | Negative        |
| HIVDNA09-04 | HIV-1 DNA PBMC          | Buffer          | 100                        | Strong Positive |
| HIVDNA09-05 | HIV-1 DNA PBMC          | Buffer          | 500                        | Strong Positive |
| HIVDNA09-06 | HIV-1 DNA PBMC          | Buffer          | 0.8                        | Weak Positive   |
| HIVDNA09-07 | HIV-1 DNA PBMC          | Buffer          | 0.16                       | Weak Positive   |
| HIVDNA09-08 | HIV-1 DNA PBMC          | Buffer          | 4                          | Positive        |

### Key to Table 2

Sample: QCMD panel sample codes for the samples distributed to participants. Sample content: viral or microbial content of the panel samples. Sample matrix: material used as a matrix in preparation of the panel samples. Sample conc.: Predefined specifications for QCMD internal purposes only. Values should not be used by participants for method comparison or as a target for individual laboratory performance assessment. Sample status: The sample status assigned to each panel sample consisting of 'Strong positive', 'Positive', 'Weak positive' or 'Negative'. Please see Appendix A for more information.

\*  $1.0 \times 10^6$  peripheral blood mononuclear cells (PBMC) positive or negative for HIV-1 pro-viral DNA.

† Phosphate buffered saline.

## 4. Programme results

### 4a. Qualitative analysis of the EQA data

The number (percentage) of correct qualitative results are presented in Table 3. Qualitative data were returned by participants as 'positive', 'negative' or 'not determined'. Not determined results were counted as incorrect for all panel samples (positive or negative).

QCMD organises datasets according to commercial and in-house technology groups, which are Conventional PCR, Real time PCR, NASBA, SDA, TMA and bDNA. Where datasets were reported as 'other' for a technology or kit method this was reviewed by the QCMD Neutral Office and assigned to an appropriate group where possible.

**Table 3: Number of correct qualitative results per panel member and technology type**

| Sample      | Sample content          | Sample conc.<br>Copies/sample | Total datasets<br>n=54 |       | PCR                             |       |                               |       |                                |       |                               |       |
|-------------|-------------------------|-------------------------------|------------------------|-------|---------------------------------|-------|-------------------------------|-------|--------------------------------|-------|-------------------------------|-------|
|             |                         |                               |                        |       | Conventional                    |       |                               |       | Real time                      |       |                               |       |
|             |                         |                               |                        |       | Commercial <sup>a</sup><br>n=13 |       | In-house <sup>b</sup><br>n=17 |       | Commercial <sup>c</sup><br>n=8 |       | In-house <sup>d</sup><br>n=16 |       |
| n           | %                       | n                             | %                      | n     | %                               | n     | %                             |       |                                |       |                               |       |
| HIVDNA09-05 | HIV-1 DNA PBMC          | 500                           | 54                     | 100.0 | 13                              | 100.0 | 17                            | 100.0 | 8                              | 100.0 | 16                            | 100.0 |
| HIVDNA09-04 | HIV-1 DNA PBMC          | 100                           | 53                     | 98.1  | 13                              | 100.0 | 16                            | 94.1  | 8                              | 100.0 | 16                            | 100.0 |
| HIVDNA09-02 | HIV-1 DNA PBMC          | 20                            | 52                     | 96.3  | 12                              | 92.3  | 17                            | 100.0 | 7                              | 87.5  | 16                            | 100.0 |
| HIVDNA09-08 | HIV-1 DNA PBMC          | 4                             | 40                     | 74.1  | 12                              | 92.3  | 12                            | 70.6  | 7                              | 87.5  | 9                             | 56.3  |
| HIVDNA09-06 | HIV-1 DNA PBMC          | 0.80                          | 29                     | 53.7  | 12                              | 92.3  | 7                             | 41.2  | 5                              | 62.5  | 5                             | 31.3  |
| HIVDNA09-07 | HIV-1 DNA PBMC          | 0.16                          | 11                     | 20.4  | 4                               | 30.8  | 2                             | 11.8  | 2                              | 25.0  | 3                             | 18.8  |
| HIVDNA09-01 | HIV-1 DNA Negative PBMC |                               | 52                     | 96.3  | 13                              | 100.0 | 16                            | 94.1  | 8                              | 100.0 | 15                            | 93.8  |
| HIVDNA09-03 | HIV-1 DNA Negative PBMC |                               | 52                     | 96.3  | 13                              | 100.0 | 16                            | 94.1  | 8                              | 100.0 | 15                            | 93.8  |

#### Key to Table 3

Sample: QCMD panel sample codes for the samples distributed to participants. Sample content: viral or microbial content of the panel samples. Sample conc.: Predefined specifications for QCMD internal purposes only. Values should not be used by participants for method comparison or as a target for individual laboratory performance assessment. Total datasets: number and percentage of datasets reporting the correct qualitative result for each panel sample. A breakdown of the results for all datasets is also provided based on technology type.

a: Roche Amplicor HIV-1 (n=13).

b: Details not presented.

c: Biocentric Generic HIV DNA Cell (n=1), Genome Diagnostics Pvt HIV-1 Real-Time PCR Kit (n=1), Roche COBAS AmpliPrep / COBAS TaqMan HIV-1 (n=6).

d: Details not presented.

## 4b. Qualitative performance scores

**Table 4: Qualitative performance scores per technology type**

| Sample      | Sample Status   | Total            |    | PCR                     |   |    |   |                       |   |    |    |                         |   |   |   |                       |   |    |    |   |   |
|-------------|-----------------|------------------|----|-------------------------|---|----|---|-----------------------|---|----|----|-------------------------|---|---|---|-----------------------|---|----|----|---|---|
|             |                 | All technologies |    | Conventional            |   |    |   |                       |   |    |    | Real time               |   |   |   |                       |   |    |    |   |   |
|             |                 | n=54             |    | Commercial <sup>a</sup> |   |    |   | In-house <sup>b</sup> |   |    |    | Commercial <sup>c</sup> |   |   |   | In-house <sup>d</sup> |   |    |    |   |   |
|             |                 | 0                | 1  | 2                       | 3 | 0  | 1 | 2                     | 3 | 0  | 1  | 2                       | 3 | 0 | 1 | 2                     | 3 | 0  | 1  | 2 | 3 |
| HIVDNA09-05 | Strong Positive | 54               | 0  | 0                       | 0 | 13 | 0 | 0                     | 0 | 17 | 0  | 0                       | 0 | 8 | 0 | 0                     | 0 | 16 | 0  | 0 | 0 |
| HIVDNA09-04 | Strong Positive | 53               | 0  | 0                       | 1 | 13 | 0 | 0                     | 0 | 16 | 0  | 0                       | 1 | 8 | 0 | 0                     | 0 | 16 | 0  | 0 | 0 |
| HIVDNA09-02 | Strong Positive | 52               | 0  | 0                       | 2 | 12 | 0 | 0                     | 1 | 17 | 0  | 0                       | 0 | 7 | 0 | 0                     | 1 | 16 | 0  | 0 | 0 |
| HIVDNA09-08 | Positive        | 40               | 0  | 14                      | 0 | 12 | 0 | 1                     | 0 | 12 | 0  | 5                       | 0 | 7 | 0 | 1                     | 0 | 9  | 0  | 7 | 0 |
| HIVDNA09-06 | Weak Positive   | 29               | 25 | 0                       | 0 | 12 | 1 | 0                     | 0 | 7  | 10 | 0                       | 0 | 5 | 3 | 0                     | 0 | 5  | 11 | 0 | 0 |
| HIVDNA09-07 | Weak Positive   | 11               | 43 | 0                       | 0 | 4  | 9 | 0                     | 0 | 2  | 15 | 0                       | 0 | 2 | 6 | 0                     | 0 | 3  | 13 | 0 | 0 |
| HIVDNA09-01 | Negative        | 52               | 0  | 0                       | 2 | 13 | 0 | 0                     | 0 | 16 | 0  | 0                       | 1 | 8 | 0 | 0                     | 0 | 15 | 0  | 0 | 1 |
| HIVDNA09-03 | Negative        | 52               | 0  | 0                       | 2 | 13 | 0 | 0                     | 0 | 16 | 0  | 0                       | 1 | 8 | 0 | 0                     | 0 | 15 | 0  | 0 | 1 |

### Key to Table 4

Sample: QCMD panel sample codes for the samples distributed to participants. Sample status: the sample status assigned to each panel sample. Please see Appendix A for more information. Total. All technologies: number of datasets awarded each score (0 to 3). A breakdown of the results for all datasets is also provided based on technology type. These data are presented graphically in Figure 1.

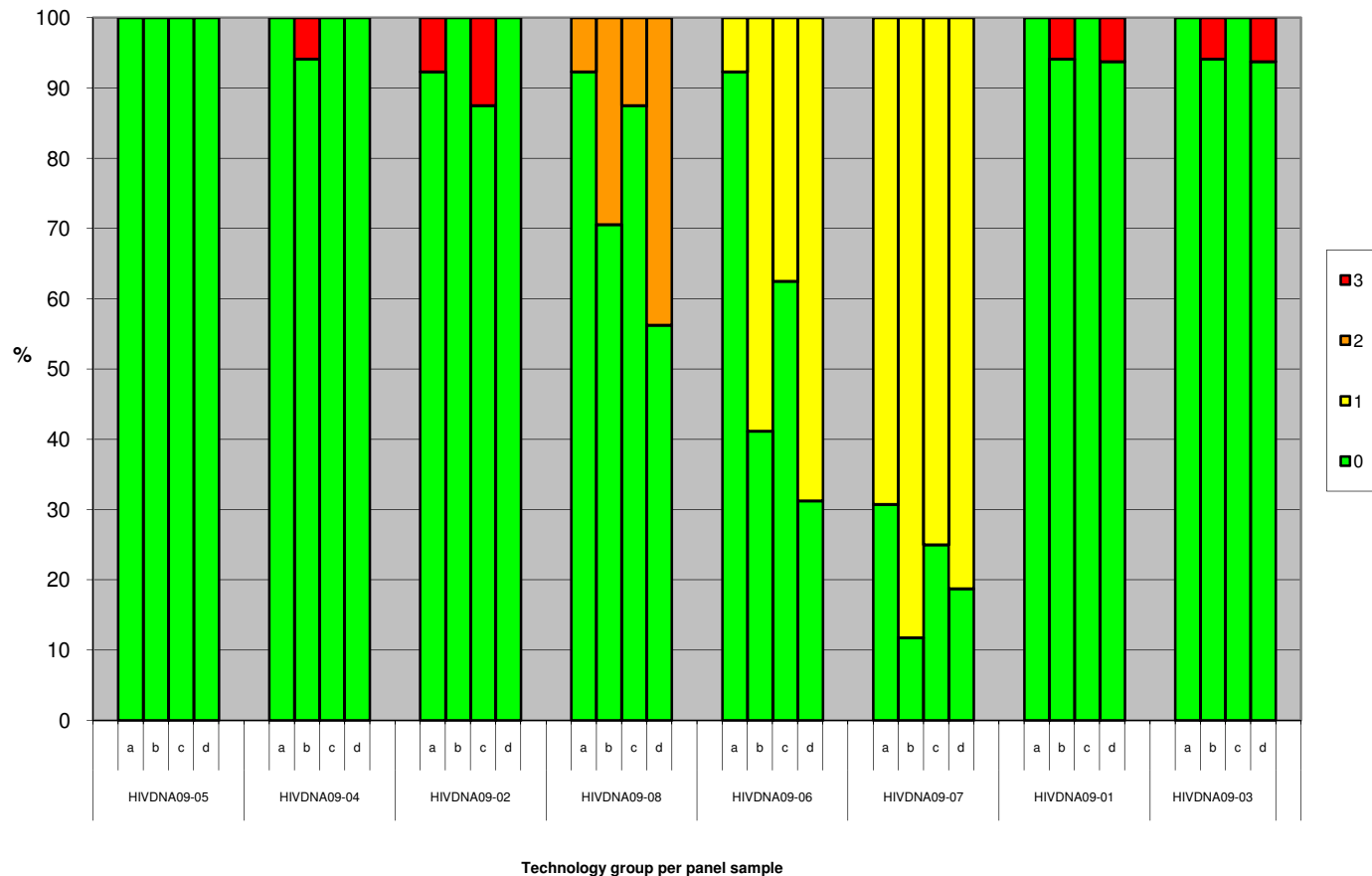
a: Roche Amplicor HIV-1 (n=13).

b: Details not presented.

c: Biocentric Generic HIV DNA Cell (n=1), Genome Diagnostics Pvt HIV-1 Real-Time PCR Kit (n=1), Roche COBAS AmpliPrep / COBAS TaqMan HIV-1 (n=6).

d: Details not presented.

**Figure 1: Percentage of qualitative performance scores per technology type**



a: Conventional Commercial PCR, b: Conventional In-house PCR, c: Real time Commercial PCR, d: Real time In-house PCR.

## 5. Comments

The number of participants in the QCMD HIVDNA EQA programmes has been consistent in recent years (51 in 2008A and 58 in 2009A).

In this year's programme there was an increase in the percentage of quantitative results submitted (from 6% in 2008A to 15% in 2009A).

There was an increase in the use of Commercial and In-house Real time PCR technologies in the 2009 distribution. Twenty-four percent of datasets reported results generated using In house Real time PCR assays in last year's programme (2008A), while 44% of datasets reported results generated using Real time PCR assays in 2009 (15% Commercial Real time PCR and 29% In house Real time PCR).

The detection rate for panel samples HIVDNA09-08 and -02 (containing approximately 4 or 20 Copies/sample) showed an overall increase between 2007 and 2009 (see table 5). However between 2008B and 2009A there was a slight decline in performance on panel samples with approximately 4 Copies/sample. The primary uses of the HIVDNA test is in infant diagnosis and current literature indicates viral load values in the range used within this EQA distribution. Hence false negative results on these samples should be taken into account in the context of a laboratory's Quality Management System and the potential use of the assays in the clinical setting.

The panel samples with a lower viral load (HIVDNA09 -07 and -06: approximately 0.16 and 0.8 Copies/sample) still appear to be challenging however there was an improvement over previous programmes. Their detection rates were: 20.4% and 53.7 % in 2009 compared to 13.0%; 30.4% in 2008B respectively.

The improvement in the rate of false positives was sustained when compared to previous distributions despite the increase in the number of participants and technologies.

**Table 5: Percentage of correct results per similar viral load panel members across the programmes**

| Sample      | Sample conc.<br>Copies/sample | % correct results |           |           |           |
|-------------|-------------------------------|-------------------|-----------|-----------|-----------|
|             |                               | HIVDNA07B         | HIVDNA08A | HIVDNA08B | HIVDNA09A |
| HIVDNA09-05 | 500                           | 85                | 100       | 100       | 100       |
| HIVDNA09-04 | 100                           | 100               | 100       | 100       | 98.1      |
| HIVDNA09-02 | 20                            | 75.0              | 90.2      | 91.3      | 96.3      |
| HIVDNA09-08 | 4                             | 55.0              | 56.9      | 82.6      | 74.1      |
| HIVDNA09-06 | 0.8                           | 35.0              | 31.4      | 30.4      | 53.7      |
| HIVDNA09-07 | 0.16                          | 10                | 3.9       | 13        | 20.4      |

### Key to Table 5

Sample: QCMD panel sample codes for the samples distributed to participants. Sample conc.: Predefined specifications for QCMD internal purposes only. Values should not be used by participants for method comparison or as a target for individual laboratory performance assessment. % correct results: % of correct qualitative results submitted by the participants over the years.

## Acknowledgements

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# Appendix A

## Assigning the sample status

QCMD uses a colour-coded scheme for scoring based on the classification of results in relation to expected or consensus results. Each panel sample is assigned a status in the EQA programme. The statuses for panel samples containing the target are 'Strong Positive', 'Positive' and 'Weak positive'. Panel samples negative for the target are assigned a 'Negative' status. The sample status is defined based on performance in the EQA programme and the expertise of the QCMD Scientific Advisory Board.

'Strong Positive': Defined as an EQA panel sample containing amounts of target agent at levels that are considered detectable by most laboratories using currently available methods. This sample produces an unequivocal positive result. 'Positive': Defined as an EQA panel sample containing amounts of target agent at levels considered detectable using the majority of the current methods available within routine clinical laboratories. This sample should produce an acceptable level of positive results within the peer group. 'Weak Positive': Defined as an EQA panel sample containing amounts of target agent at a level that is known to be problematic for current laboratory methods in routine clinical use. 'Negative': Defined as an EQA panel sample with a common matrix to other test samples but containing no target agent and producing an unequivocal negative result.

## Scoring system for qualitative EQA data

The scores awarded for qualitative EQA data were based on the sample status (see Section 3). The scoring system is represented in the following table, where 0 is 'highly satisfactory' and 3 is 'highly unsatisfactory'. Colour has been included as an extra visual aid.

### Scoring system based on the assigned sample status

| Sample status   | Participant's result |                |          |
|-----------------|----------------------|----------------|----------|
|                 | Negative             | Not determined | Positive |
| Strong Positive | 3                    | 3              | 0        |
| Positive        | 2                    | 2              | 0        |
| Weak Positive   | 1                    | 1              | 0        |
| Negative        | 0                    | 3              | 3        |